

## WEST Search History

DATE: Wednesday, October 22, 2003

**Set Name Query**  
side by side

**Hit Count Set Name**  
result set

*DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ*

L5 modified phytase and (27 or 66 or 140 or 141 or 205 or 274 or 277 or  
282 or 340)

9 L5

*DB=USPT; PLUR=YES; OP=ADJ*

L4 modified phytase and (27 or 66 or 140 or 141 or 205 or 274 or 277 or  
282 or 340)

3 L4

L3 phytase and position 27

19 L3

L2 phytase and amino acid 27

0 L2

L1 6391605

2 L1

END OF SEARCH HISTORY

=> file medline caplus biosis biotechds scisearch embase		
COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 15:46:18 ON 22 OCT 2003

FILE 'CAPLUS' ENTERED AT 15:46:18 ON 22 OCT 2003  
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FILE 'EMBASE' ENTERED AT 15:46:18 ON 22 OCT 2003  
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=> s modified phytase and (27 or 66 or 140 or 141 or 205 or 274 or 277 or 282 or 340)  
 L1 0 MODIFIED PHYTASE AND (27 OR 66 OR 140 OR 141 OR 205 OR 274 OR  
 277 OR 282 OR 340)

=> s (modified phytase? or phytase variant? or phytase mutant?) and (27 or 66 or 140 or  
 L2 5 (MODIFIED PHYTASE? OR PHYTASE VARIANT? OR PHYTASE MUTANT?) AND  
 (27 OR 66 OR 140 OR 141 OR 205 OR 274 OR 277 OR 282 OR 340)

=> dup rem l2  
 PROCESSING COMPLETED FOR L2  
 L3 1 DUP REM L2 (4 DUPLICATES REMOVED)

=> d l3 ibib ab

L3 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2002215248 MEDLINE  
 DOCUMENT NUMBER: 21914102 PubMed ID: 11916711  
 TITLE: Engineering of phytase for improved activity at low pH.  
 AUTHOR: Tomschy Andrea; Brugger Roland; Lehmann Martin; Svendsen  
 Allan; Vogel Kurt; Kostrewa Dirk; Lassen Soren F; Burger  
 Dominique; Kronenberger Alexandra; van Loon Adolphus P G M;  
 Pasamontes Luis; Wyss Markus  
 CORPORATE SOURCE: Biotechnology Department, Roche Vitamins, Ltd., 4070 Basel,  
 Switzerland.  
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2002 Apr) 68 (4)  
 1907-13.  
 Journal code: 7605801. ISSN: 0099-2240.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200207  
 ENTRY DATE: Entered STN: 20020416  
 Last Updated on STN: 20020713  
 Entered Medline: 20020712

AB For industrial applications in animal feed, a phytase of interest must be  
 optimally active in the pH range prevalent in the digestive tract.  
 Therefore, the present investigation describes approaches to rationally  
 engineer the pH activity profiles of *Aspergillus fumigatus* and consensus  
 phytases. Decreasing the negative surface charge of the *A. fumigatus* Q27L  
**phytase mutant** by glycinamidylation of the surface

carboxy groups (of Asp and Glu residues) lowered the pH optimum by ca. 0.5 unit but also resulted in 70 to 75% inactivation of the enzyme. Alternatively, detailed inspection of amino acid sequence alignments and of experimentally determined or homology modeled three-dimensional structures led to the identification of active-site amino acids that were considered to correlate with the activity maxima at low pH of *A. niger* NRRL 3135 phytase, *A. niger* pH 2.5 acid phosphatase, and *Peniophora lycii* phytase. Site-directed mutagenesis confirmed that, in *A. fumigatus* wild-type phytase, replacement of Gly-277 and Tyr-282 with the corresponding residues of *A. niger* phytase (Lys and His, respectively) gives rise to a second pH optimum at 2.8 to 3.4. In addition, the K68A single mutation (in both *A. fumigatus* and consensus phytase backbones), as well as the S140Y D141G double mutation (in *A. fumigatus* phytase backbones), decreased the pH optima with phytic acid as substrate by 0.5 to 1.0 unit, with either no change or even a slight increase in maximum specific activity. These findings significantly extend our tools for rationally designing an optimal phytase for a given purpose.

=> d his

(FILE 'HOME' ENTERED AT 15:45:40 ON 22 OCT 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 15:46:18 ON 22 OCT 2003

L1	0 S MODIFIED PHYTASE AND (27 OR 66 OR 140 OR 141 OR 205 OR 274 OR
L2	5 S (MODIFIED PHYTASE? OR PHYTASE VARIANT? OR PHYTASE MUTANT?) AN
L3	1 DUP REM L2 (4 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	50.90	51.11

STN INTERNATIONAL LOGOFF AT 15:48:19 ON 22 OCT 2003

## Print

☐ 1. Document ID: US 6579975 B1

Jun 17, 2003

DOCUMENT-IDENTIFIER: US 6579975 B1

DATE-ISSUED: June 17, 2003

## INVENTOR - INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Inzlingen			DE

US-CL-CURRENT: 536/23.2; 435/195, 435/196, 435/252.3, 435/320.1, 435/325, 435/69.1

[illegible]

☐ 2. Document ID: US 6391605 B1

May 21, 2002

DOCUMENT-IDENTIFIER: US 6391605 B1

**\*\* See image for Certificate of Correction \*\***

DATE-ISSUED: May 21, 2002

## INVENTOR - INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kostrewa; Dirk	Freiburg			DE
Pasamontes; Luis	Trimbach			CH
Tomschy; Andrea	Grenzach-Wyhlen			DE
van Loon; Adolphus	Rheinfelden			CH
Vogel; Kurt	Basel			CH
Wyss; Markus	Liestal			CH

US-CL-CURRENT: 435/196; 424/94.6

[illegible]

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Terms	Documents
6391605	2

**Display Format:**[CIT](#)[Change Format](#)[Previous Page](#)[Next Page](#)

**WEST****End of Result Set**☐ **Generate Collection** **Print**

L1: Entry 2 of 2

File: USPT

May 21, 2002

US-PAT-NO: 6391605DOCUMENT-IDENTIFIER: US 6391605 B1**\*\* See image for Certificate of Correction \*\***

TITLE: Modified phytases

DATE-ISSUED: May 21, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kostrewa; Dirk	Freiburg			DE
Pasamontes; Luis	Trimbach			CH
Tomschy; Andrea	Grenzach-Wyhlen			DE
van Loon; Adolphus	Rheinfelden			CH
Vogel; Kurt	Basel			CH
Wyss; Markus	Liestal			CH

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Roche Vitamins Inc.	Parsippany	NJ			02

APPL-NO: 09/ 044718 [PALM]

DATE FILED: March 19, 1998

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97810175	March 25, 1997

INT-CL: [07] C12 N 9/16, A61 K 38/46

US-CL-ISSUED: 435/196; 424/94.6

US-CL-CURRENT: 435/196; 424/94.6

FIELD-OF-SEARCH: 435/196, 424/94.6

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

☐ **Search Selected** ☐ **Search ALL**

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>5436156</u>	July 1995	Van Gorcom et al.	
<input type="checkbox"/>	<u>5443979</u>	August 1995	Vanderbeke et al.	
<input type="checkbox"/>	<u>5863533</u>	January 1999	Van Gorcom et al.	

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
1 492 060	March 1969	DE	
0 035 204	September 1981	EP	
299 108	January 1989	EP	
420 358	April 1991	EP	
0 422 697	April 1991	EP	
619 369	October 1994	EP	
684 313	November 1995	EP	
0 758 018	February 1997	EP	
747 483	May 1997	EP	
0 897 010	February 1999	EP	
0 897 985	February 1999	EP	
98104858	February 1999	EP	
WO 91/14773	October 1991	WO	
93/16175	August 1993	WO	
94/03612	February 1994	WO	
WO 95/00662	January 1995	WO	
WO 98/54980	December 1998	WO	

## OTHER PUBLICATIONS

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ART-UNIT: 1652

PRIMARY-EXAMINER: Achutamurthy; Ponnathapu

ASSISTANT-EXAMINER: Tung; Peter

ATTY-AGENT-FIRM: Bryan Cave LLP

ABSTRACT:

A process for the production of a modified phytase with a desired property improved over the property of the corresponding unmodified phytase is disclosed, as well as modified phytases, polynucleotides encoding modified phytases, and animal feed including modified phytases.

24 Claims, 97 Drawing figures



**WEST**☐ Generate Collection☐ Print

L3: Entry 4 of 19

File: USPT

May 27, 2003

US-PAT-NO: 6569659

DOCUMENT-IDENTIFIER: US 6569659 B1

TITLE: Phytase polypeptides

DATE-ISSUED: May 27, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lassen; Soren Flensted	Copenhagen			DK
Bech; Lisbeth	Hillerod			DK
Ohmann; Anders	Bronshoj			DK
Breinholt; Jens	Bagsvaerd			DK
Fuglsang; Claus Crone	Niva			DK
Ostergaard; Peter Rahbek	Virum			DK

US-CL-CURRENT: 435/196; 435/183, 435/252.3, 435/320.1, 435/69.1, 536/23.1, 536/23.2, 536/23.6, 536/24.33

## CLAIMS:

What is claimed is:

1. An isolated polynucleotide which encodes a polypeptide having phytase activity and hybridizes under high stringency conditions with any of the DNA sequences of SEQ ID NO:21, 25, 27 or 29, or with any of the DNA sequences cloned into plasmid pYES 2.0 present in Escherichia coli DSM 11313, 11842, 11843, or 11844, wherein the high stringency conditions are defined as prehybridization in a solution of 5.times.SSC, 5.times.Denhardt's solution, 0.5% SDS and 100 micro-g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approximately 45.degree. C., followed by washing twice for 30 minutes in 2.times.SSC, 0.5% SDS at at least 70.degree. C.
2. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence of residues -26-427, -2-427, 1-427, 2-427, or 5-427 of SEQ ID NO:22.
3. The polynucleotide of claim 1, which is (a) a nucleic acid comprising nucleotides 17-1375, 89-1375, 95-1375, 98-1375, or 107-1375 of SEQ ID NO:21; (b) the DNA sequence cloned into plasmid pYES 2.0 present in Escherichia coli DSM 11313; or  
a fragment thereof that encodes a polypeptide having phytase activity.
4. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence of residues -19-423, 1-423, or 2-423 of SEQ ID NO:26.
5. The polynucleotide of claim 1, which is (a) a nucleic acid comprising nucleotides 58-1383, 115-1383, or 118-1383 of SEQ ID NO 25; (b) the DNA sequence cloned into plasmid pYES 2.0 present in Escherichia coli 11842; or

a fragment thereof that encodes a polypeptide having phytase activity.

6. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence of residues -19-423, 1-423, or 6-423 of SEQ ID NO 28.

7. The polynucleotide of claim 1, which is (a) a nucleic acid comprising nucleotides 48-1373, 105-1373, or 120-1373 of SEQ ID NO 27; (b) the DNA sequence cloned into plasmid pYES 2.0 present in Escherichia coli DSM 11843; or a fragment thereof that encodes a polypeptide having phytase activity.

8. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence of residues -17-426, 1-426, or 14-426 of SEQ ID NO 30.

9. The polynucleotide of claim 1, which is (a) a nucleic acid sequence comprising nucleotides 79-1407, 130-1407, and 169-1407 of SEQ ID NO 29; (b) the DNA sequence cloned into plasmid pYES 2.0 present in Escherichia coli DSM 11844; or

a fragment thereof that encodes a polypeptide having phytase activity.

10. The polynucleotide of claim 1, wherein the polypeptide is a basidiomycete polypeptide.

11. A vector comprising the polynucleotide of claim 1.

12. A host cell comprising the polynucleotide of claim 1.

13. A process for preparing a polypeptide having phytase activity, comprising (a) culturing the host cell of claim 12 under conditions permitting the production of the polypeptide; and (b) recovering the polypeptide from the culture broth.

14. An isolated polynucleotide which encodes a polypeptide having phytase activity and which is at least 90% homologous to any of SEQ ID NO:21, 25, 27 or 29.

15. The polynucleotide of claim 14 which is at least 95% homologous to any of SEQ ID NO:21, 25, 27 or 29.

16. The polynucleotide of claim 15 which is at least 97% homologous to any of SEQ ID NO:21, 25, 27 or 29.

17. The polynucleotide of claim 14, wherein the polypeptide is a basidiomycete polypeptide.

18. A vector comprising the polynucleotide of claim 14.

19. A host cell comprising the polynucleotide of claim 14.

20. A process for preparing a polypeptide having phytase activity, comprising (a) culturing the host cell of claim 19 under conditions permitting the production of the polypeptide; and (b) recovering the polypeptide from the culture broth.

21. The polynucleotide of claim 1, wherein the high stringency conditions are defined as prehybridization in a solution of 5 x SSC, 5 x Denhardt's solution, 0.5% SDS and 100 micro-g/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution for 12 hours at approximately 45.degree. C, followed by washing twice for 30 minutes in 2 x SSC, 0.5% SDS at at least 75.degree. C.

**WEST**[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 10 of 19 returned.**☐ 1. Document ID: US 6623948 B1

L3: Entry 1 of 19

File: USPT

Sep 23, 2003

US-PAT-NO: 6623948

DOCUMENT-IDENTIFIER: US 6623948 B1

TITLE: Nucleic acid sequences encoding alkaline alpha-amylases

DATE-ISSUED: September 23, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Outtrup; Helle	Vaerlose			DK
Hoeck; Lisbeth Hedegaard	Frørup			DK
Nielsen; Bjarne Ronfeldt	Virum			DK
Borchert; Torben Vedel	Copenhagen			DK
Nielsen; Vibeke Skovgaard	Bagsvaerd			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsvaerd			DK
Svendsen; Allan	Birkerød			DK
Andersen; Carsten	Vaerlose			DK

US-CL-CURRENT: 435/202; 435/252.3, 435/254.11, 435/320.1, 435/325, 435/419,  
536/23.1, 536/23.2, 536/23.7

Full	Title	Citation	Front	Revised	Classification	Date	Reference	Sequences	Attachments	Claims	Footc
Draw Data	Image										

☐ 2. Document ID: US 6617026 B2

L3: Entry 2 of 19

File: USPT

Sep 9, 2003

US-PAT-NO: 6617026

DOCUMENT-IDENTIFIER: US 6617026 B2

TITLE: Particles containing active in visco-elastic liquids

DATE-ISSUED: September 9, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bach; Poul	Birkerød slashed.d			DK

US-CL-CURRENT: 428/402; 427/212, 427/213, 427/213.31, 428/402.2, 428/403, 428/407

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	Footc
Draw Desc	Image										

☐ 3. Document ID: US 6608018 B1

L3: Entry 3 of 19

File: USPT

Aug 19, 2003

US-PAT-NO: 6608018

DOCUMENT-IDENTIFIER: US 6608018 B1

TITLE: Polypeptides having branching enzyme activity and nucleic acids encoding same

DATE-ISSUED: August 19, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shinohara; Mari L.	Brookline	MA		

US-CL-CURRENT: 510/392; 435/193

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Footc
Draw Desc	Image									

☐ 4. Document ID: US 6569659 B1

L3: Entry 4 of 19

File: USPT

May 27, 2003

US-PAT-NO: 6569659

DOCUMENT-IDENTIFIER: US 6569659 B1

TITLE: Phytase polypeptides

DATE-ISSUED: May 27, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lassen; Soren Flensted	Copenhagen			DK
Bech; Lisbeth	Hillerod			DK
Ohmann; Anders	Bronshoj			DK
Breinholt; Jens	Bagsvaerd			DK
Fuglsang; Claus Crone	Niva			DK
Ostergaard; Peter Rahbek	Virum			DK

US-CL-CURRENT: 435/196; 435/183, 435/252.3, 435/320.1, 435/69.1, 536/23.1, 536/23.2, 536/23.6, 536/24.33

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Footc
Draw Desc	Image									

☐ 5. Document ID: US 6528298 B1

L3: Entry 5 of 19

File: USPT

Mar 4, 2003

US-PAT-NO: 6528298  
DOCUMENT-IDENTIFIER: US 6528298 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: March 4, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerod			DK
Borchert; Torben Vedel	Copenhagen			DK
Bisgard-Frantzen; Henrik	Bagsvaerd			DK
Outtrup; Helle	Ballerup			DK
Nielsen; Bjarne Ronfeldt	Virum			DK
Nielsen; Vibeke Skovgaard	Bagsoe butted.rd			DK
Hedegaard; Lisbeth	Skodsborg			DK

US-CL-CURRENT: 435/202; 435/183, 435/200, 435/201, 435/252.3, 435/320.1, 435/69.1,  
536/23.2, 536/23.7

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Print
Draw Data	Image									

☐ 6. Document ID: US 6524827 B2

L3: Entry 6 of 19

File: USPT

Feb 25, 2003

US-PAT-NO: 6524827  
DOCUMENT-IDENTIFIER: US 6524827 B2

TITLE: 2,6-.beta.-D-fructan hydrolase enzyme and processes for using the enzyme

DATE-ISSUED: February 25, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moller; Soren	Holte			DK
Johansen; Charlotte	Holte			DK
Schafer; Thomas	Farum			DK
Ostergaard; Peter Rahbek	Virum			DK
Hoeck; Lisbeth Hedegaard	Skodsborg			DK

US-CL-CURRENT: 435/74; 435/183, 435/252.3, 435/252.33, 435/320.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Print
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☐ 7. Document ID: US 6521434 B2

L3: Entry 7 of 19

File: USPT

Feb 18, 2003

US-PAT-NO: 6521434

DOCUMENT-IDENTIFIER: US 6521434 B2

TITLE: Nucleic acids encoding polypeptides having haloperoxidase activity

DATE-ISSUED: February 18, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Danielsen; Steffen	Copenhagen			DK
Schneider; Palle	Ballerup			DK

US-CL-CURRENT: 435/192; 435/252.3, 435/320.1, 435/911, 530/350, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Full
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☐ 8. Document ID: US 6511835 B1

L3: Entry 8 of 19

File: USPT

Jan 28, 2003

US-PAT-NO: 6511835

DOCUMENT-IDENTIFIER: US 6511835 B1

TITLE: Nucleic acids encoding polypeptides having haloperoxidase activity

DATE-ISSUED: January 28, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Danielsen; Steffen	Copenhagen			DK
Schneider; Palle	Lynge			DK

US-CL-CURRENT: 435/192; 435/252.3, 435/320.1, 435/911, 530/350, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Full
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☐ 9. Document ID: US 6509181 B1

L3: Entry 9 of 19

File: USPT

Jan 21, 2003

US-PAT-NO: 6509181

DOCUMENT-IDENTIFIER: US 6509181 B1

TITLE: Polypeptides having haloperoxide activity

DATE-ISSUED: January 21, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Danielsen; Steffen	Copenhagen			DK
Schneider; Palle	Lynge			DK

US-CL-CURRENT: [435/192](#); [435/252.3](#), [435/320.1](#), [435/911](#), [530/350](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Print
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☐ 10. Document ID: US 6506586 B2

L3: Entry 10 of 19

File: USPT

Jan 14, 2003

US-PAT-NO: 6506586

DOCUMENT-IDENTIFIER: US 6506586 B2

TITLE: Nucleic acids encoding polypeptides having haloperoxidase activity

DATE-ISSUED: January 14, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Palle	Lynge			DK
Danielsen; Steffen	Copenhagen			DK

US-CL-CURRENT: [435/192](#); [435/252.3](#), [435/320.1](#), [435/911](#), [530/350](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Print
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phytase and position 27	19

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May 21, 2002

US-PAT-NO: 6391605

DOCUMENT-IDENTIFIER: US 6391605 B1

**\*\* See image for Certificate of Correction \*\***TITLE: Modified phytases

DATE-ISSUED: May 21, 2002

## INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/196; 424/94.6

## CLAIMS:

What is claimed is:

1. A modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.
2. A modified *Aspergillus fumigatus* phytase according to claim 1, further comprising an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.
3. A modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the modified *Aspergillus fumigatus* phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations thereof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an *Aspergillus niger* phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.
4. A modified phytase according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.
5. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ala.
6. A modified phytase according to claim 4 wherein the amino acid sequence of



SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Val.

7. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Leu.

8. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ile.

9. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Thr.

10. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Asn.

11. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Gly.

12. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.

13. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.

14. A modified phytase according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

15. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.

16. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.

17. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.

18. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.

19. A modified phytase according to claim 3 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.

20. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.

21. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.

22. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.

23. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.

24. A food or feed composition comprising a modified phytase of claim 1.

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File: USPT

Mar 21, 2000

US-PAT-NO: 6039942

DOCUMENT-IDENTIFIER: US 6039942 A

TITLE: Phytase polypeptides

DATE-ISSUED: March 21, 2000

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INT-CL: [07] A61 K 38/46, C12 N 9/14, C12 N 1/20, C12 N 15/00

US-CL-ISSUED: 424/94.6; 435/195, 435/196, 435/252.3, 435/320.1, 435/18, 536/23.2, 536/23.7, 530/300, 530/350

US-CL-CURRENT: 424/94.6; 435/18, 435/195, 435/196, 435/252.3, 435/320.1, 530/300, 530/350, 536/23.2, 536/23.7

FIELD-OF-SEARCH: 424/94.6, 435/195, 435/18, 435/196, 435/252.3, 435/320.1, 536/23.2, 536/23.7, 530/300, 530/350

PRIOR-ART-DISCLOSED:

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 684 313	November 1995	EP	

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C. McElhinney et al., (1993) Mycol. Res. 97(6):725-732.  
PIR Database No. Pirl:Jn0482, Accesssion No. JN0482.

ART-UNIT: 162

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L5: Entry 5 of 9

File: PGPB

Sep 12, 2002

PGPUB-DOCUMENT-NUMBER: 20020127218  
PGPUB-FILING-TYPE: new  
DOCUMENT-IDENTIFIER: US 20020127218 A1

TITLE: Phytase variants

PUBLICATION-DATE: September 12, 2002

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US-CL-CURRENT: 424/94.6; 435/196, 435/320.1, 435/325, 435/69.1, 536/23.2

## CLAIMS:

1. A phytase variant which, when aligned according to FIG. 1, is amended as compared to a model phytase in at least one of the following positions, using the position numbering corresponding to P<sub>lycii</sub>: 24; 27; 31; 33; 39; 40; 41; 42; 43; 44; 45; 46; 47; 49; 51; 56; 58; 59; 61; 62; 68; 69; 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 88; 90; 102; 115; 116; 117; 118; 119; 120; 121; 122; 123; 124; 125; 126; 127; 128; 132; 143; 148; 149; 150; 151; 152; 153; 154; 155; 156; 157; 158; 159; 160; 161; 162; 163; 170f; 170g; 171; 172; 173; 184; 185; 186; 187; 187a; 190; 191; 192; 193; 194; 195; 198; 199; 200; 201; 201a; 201b; 201c; 201d; 201e; 201f; 202; 203; 203a; 204; 205; 211; 215; 220; 223; 228; 232; 233; 234; 235; 236; 237; 238; 239; 242; 243; 244; 246; 251e; 253; 256; 260; 264; 265; 267; 270; 271; 272; 273; 274; 275; 276; 277; 278; 279; 280; 283; 285; 287; 288; 292; 293; 302; 304; 332; 333; 334; 335; 336; 337; 338; 339; 340; 341; 342; 343; 348; 349; 352; 360; 362; 364; 365; 366; 367; 368; 369; 370; 371; 372; 373; 374; 375; 376; 383k; 387; 393; 394; 396; 404; 409; 411; 412; 413; 417; 421; 431:

2. A phytase variant which, when aligned according to FIG. 1, comprises at least one of the following amendments as compared to a model phytase, using the position numbering corresponding to the phytase of P<sub>lycii</sub>: 24C; 27P; 31Y; 33C; 39H,S,Q; 40L,N; 42S,G; 43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,- R,S,T,V,W,Y; 44N; 45D,S; 47Y,F; 49P; 51E,A,R; 56P; 58D,K,A; 59G; 61R; 62V,I; 69Q; 75W,F; 78D,S; 79G; 80K,A; 81A,G,Q,E; 82T; 83A,I,K,R,Q; 84I,Y,Q,V; 88I; 90R,A; 102Y; 115N; 116S; 118V,L; 119E; 120L; 122A; 123N,Q,T; 125M,S; 126H,S,V; 127Q,E,N; 128A,S,T; 132F,I,L; 143N; 148V,I; 151A,S; 152G; 153D,Y; 154D,Q,S,G; 157V; 158D,A; 159T; 160A,S; 161T,N; 162N; 163W; 170fH; 170gA; 171N; 172P; 173Q,S; 184Q,S,P; 185S; 186A,E,P; 187A; 187aS; 190A,P; 193S; 194S,T; 195T,V,L; 198A,N,V; 200G,V; 201D,E; 201a(); 201b(); 201c(); 201d(); 201e(); 201f(); 201eT; 202S,A; 203R,K,S; 203aV,T; 204Q,E,S,A,V; 205E; 211L,V; 215A,P; 220L,N; 223H,D; 228N; 232T; 233E; 235Y,L,T; 236Y,N; 237F; 238L,M; 242P,S; 244D;

246V; 251eE,Q; 253P; 256D; 260A,H; 264R,I; 265A,Q; 267D; 270Y,A,L,G; 271D,N; 273D,K; 275F,Y; 278T,H; 280A,P; 283P; 287A,T; 288L,I,F; 292F,Y; 293A,V; 302R,H; 304P,A; 332F; 336S; 337T,G,Q,S; 338I; 339V,I; 340P,A; 343A,S,F,I,L; 348Y; 349P; 352K; 360R; 362P; 364W,F; 365V,L,A,S; 366D,S,V; 367A,K; 368K; 369I,L; 370V; 373A,S; 374S,A; 375H; 376M; 383kQ,E; 387P; 393V; 396R; 404A,G; 409R; 411K,T; 412R; 417E,R; 421F,Y; 431E.

3. The phytase variant of any of claims 1 or 2, which is derived from an ascomycete phytase.

4. The phytase variant of claim 3, which is derived from an *Aspergillus* phytase.

5. The phytase variant of claim 4, wherein the model phytase is a strain of *Aspergillus niger*, *Aspergillus ficuum*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Aspergillus terreus*.

6. The phytase variant of claim 5 wherein the model phytase is *Aspergillus nidulans* DSM 9743; or any of the following strains of *Aspergillus terreus*: CBS 116.46, DSM 9076, CBS 220.95.

7. The phytase variant of claim 6 wherein the model phytase is the *Aspergillus nidulans* phytase sequence shown in FIG. 10; or the *Aspergillus terreus* phytase sequence shown in FIG. 12.

8. The phytase variant of claim 3 wherein the model phytase is a strain of *Thermomyces lanuginosus*, *Talaromyces thermophilus*, or *Myceliophthora thermophila*.

9. The phytase variant of claim 8 wherein the model phytase is *Thermomyces lanuginosus* CBS 586.94; or any of the following strains of *Talaromyces thermophilus*: ATCC 20186, ATCC 74338; or any of the following strains of *Myceliophthora thermophila*: ATCC 34625, ATCC 74340.

10. The phytase variant of claim 9 wherein the model phytase is the *Thermomyces lanuginosus* phytase sequence shown in FIG. 14; or the *Talaromyces thermophilus* sequence shown in FIG. 13; or the *Myceliophthora thermophila* phytase sequence shown in FIG. 7.

11. The phytase variant of claim 3 wherein the model phytase is an ascomycete consensus phytase sequence.

12. The phytase variant of any of claims 1 or 2, which is derived from a basidiomycete phytase.

13. The phytase variant of claim 12, wherein the model phytase is a strain of *Paxillus involutus*, *Trametes pubescens*, *Agrocybe pediades*, or *Peniophora lycii*.

14. The phytase variant of claim 13 wherein the model phytase is *Trametes pubescens* CBS 100232 or *Paxillus involutus* CBS 100231.

15. The phytase variant of claim 14 wherein the model phytase is the *Trametes pubescens* phytase sequence of FIG. 4 or either of the *Paxillus involutus* phytase sequences of FIGS. 2 and 3.

16. The phytase variant according to any of claims 1 or 2, which comprises at least one of the following amendments: R24C; V27P; H39Q,S; L40N; G42S; Q43A,C,D,E,F,G,H,I,K,L,M,N,P,R,S,T,V,W,Y; Y44N; A45D,S; F47Y; S49P; A51E,R; V56P; A58D,K; V62I; S69Q; Y75W,F; D78S; S79G; K80A; G81A,Q,E; K82T; K83A,I,R,Q; Y84Q,I,V; E90R,A; D115N; D116S; T118V,L; P119E; F120L; E122A; Q123N,T; L125S,M; V126H,S; N127Q,E; S128A,T; F132I,L; I148V; S151A; S153D,Y; S154Q,D,G; I157V; A158D; S159T; G160A,S; K161T,N; K162N; F163W; R170fH; Q171N; G173Q,S; S184P,Q; E185S; A186E,P; S187A; T190P,A; P193S; G194S,T; T195V,L; V198A,N; E200G,V; D201E; S201d(); E201e(),T; L:201f(); preferably all three deletions; A202S; D203R,K,S; D203aV,T; V204Q,E,S,A; T211L,V; S215AP; L220N; D223H; T228N; T235Y,L; Y236N, L237F; M238L; S242P; I246V; K251eE,Q; H260A; I264R; N265Q,A; Q270Y,A,L,G; S271D,N; K273D; Y275F; H278T; A280P; T287A; Q288L,I,F; Y292F; A293V; H302R; P304A; N336S; G337S,T,Q; I339V;

S340P,A; F343A,S,F,I,L; N349P; N360R; T362P; F364W; S365V,L,A; S366D,V; A367K; W368K; T369I,L; A373S: S374A; R375H; L376M; Q383kE; P404A,G; T411K; R417E; F421Y; A431E.

17. The phytase variant of claim 16, the model phytase of which is an *Aspergillus* derived phytase, preferably derived from *Aspergillus ficuum* or *Aspergillus niger*.

18. The phytase variant of claim 17, the model phytase of which is a phytase derived from either of *Aspergillus ficuum* (*niger*) NRRL 3135, *Aspergillus niger* ATCC 9142, or *Aspergillus niger* ATCC 74337.

19. The phytase variant of claim 18, the model phytase of which is the *Aspergillus ficuum* phytase sequence of FIG. 11.

20. The phytase variant according to any of claims 1 or 2, which phytase variant comprises at least one of the following amendments: A24C; V27P; H39,S,Q; L40N; G42S; Q43C,D,E,F,H,K,M,P,R,S,W,Y; Y44N; S45D; F47Y; S49P; E51A,R; L56P; K58D,A; D59G; I62V; S69Q; Y75W,F; S78D; S79G; K80A; S81A,G,Q,E; K82T; K83A,I,Q,R; Y84Q,V,I; V88K; A90R; F102Y; D115N; D116S; T118V,L; P119E; F120L; E122A; Q123N,T; L125S,M; V126H,S; N127Q,E; S128A,T; F132,I,L; S143N; I148V; S151A; S153D,Y; D154Q,S,G; I157V; A158D; S159T; G160A,S; E161T,N; K162N; F163W; G170fH; ()171N; N173Q,S; T172P; P184Q,S; E185S; S186A,E,P; E187A; T187aS; T190P,A; G194S,T; V195L,T; K198A,N,V; E200G,V; A201D,E; S201d(); Q201e(),T; L201f(); preferably all three deletions; G202S,A; D203R,K,S; E203aV,T; V204Q,E,S,A; A205E; L211V; A220L,N; H223D; T228N; E232T; D233E; V235Y,L,T; V236Y,N; L237F; M238L; C242P,S; T246V; Q251eE,Q; Q256D; H260A; K264R,I; K265Q,A; N267D; Q270Y,A,L,G; S271D,N; G273D,K; Y275F; Y278T,H; A280P; A287T; Q288L,I,F; F292Y; T293A,V; R302H; P304A; F332F; N336S; S337T,G,Q; M338I; V339I; S340P,A; F343A,S,I,L; N349P; E352K; S360R; K362P; Y364W,F; S365V,L,A; A366D,V,S; S367A,K; W368K; V369I,L; G373S,A; R375H; A376M; K383kQ,E; D404A,G; K411T; I393V; L412R; K417E,R; W421F,Y; G431E.

21. The phytase variant of claim 20, which is derived from an *Aspergillus* phytase, preferably using a model phytase derived from *Aspergillus fumigatus*.

22. The phytase variant of claim 21, the model phytase of which is a phytase derived from either of the following strains of *Aspergillus fumigatus*: ATCC 13073, ATCC 32722, ATCC 58128, ATCC 26906 or ATCC 32239.

23. The phytase variant of claim 22, the model phytase of which is the *Aspergillus fumigatus* phytase sequence of FIG. 8.

24. The phytase variant according to any of claims 1 or 2, which phytase variant comprises at least one of the following amendments: 24C; V27P; H39S,Q; L40N; G42S; Q43A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y; Y44N; S45D; Y47F; S49P; E51A,R; V56P; D58K,A; D59G; V62I; S69Q; Y75W,F; S78D; S79G; K80A; S81A,G,Q,E; K82T; A83I,Q,K,R; Y84,Q,I,V; A90R; D115N; D116S; T118V,L; F119E; P120L; E122A; N123Q,T; M125S; V126H,S; N127Q,E; S128A,T; Y132F,I,L; K143N; I148V; S151A; S153D,Y; D154Q,S,G; I157V; A158D; S159T; A160S; E161T,N; K162N; F163W; G170fH; S170gA; Q171N; H173Q,S; P184Q,S; E185S; G186A,E,P; S187A; G187aS; T190P,A; H193S; G194S,T; T195V,L; A198N,V; E200G,V; D201E; S201d(); E201e(),T; L201f(); preferably all three; G202S,A; D203R,K,S; D203aV,T; V204Q,S,A,E; L211V; A215P; L220N; D223H, T228N; E232T; D233E; V235Y,L,T; Y236N; L237F; M238L; P242S; E244D; E251e,Q; A256D; H260A; R264I; Q265A; Q270Y,A,L,G; S271D,N; G273D,K; Y275F; Y278T,H; A280P; A287T; Q288L,I,F; F292Y; A293V; R302H; P304A; N336S; S337T,Q,G; M338I; I339V; S340P,A; F343A,S,I,L; N349P; A352K; S360R; E362P; Y364W,F; S365V,L,A; A366D,V,S; S367K,A; W368K; T369I,L; G373S,A; A374S; R375H; A376M; Q383kE; A404G; K411T; E417R; F421Y; A431E.

25. The phytase variant of claim 24, the model phytase of which is an ascomycete consensus phytase.

26. The phytase variant of claim 25, the model phytase of which is the ascomycetes consensus sequence "conphys" of FIG. 9.

27. The phytase variant according to any of claims 1 or 2, which phytase variant comprises at least one of the following amendments: V24C; F27P; ()31Y; F33C;

D39H,S,Q; S40L,N; A42S,G; A43C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T- ,V,W,Y; Y44N; T45D,S; Y47F; Q51E,A,R; K58D,A; K61R; I62V; F75W; S78D; A80K; G81A,Q,E; R83A,I,Q,K; I84Y,Q,V; V88I; K90R,A; L102Y; D115N; D116S; V118L; P119E; F120L; L123N,T,Q; S125M; S126H,V; Q127E,N; A128S,T; T132F,I,L; E143N; V148I; S151A; S152G; S153D,Y; N154D,Q,S,G; D158A; S159T; A160S; T161N; ()170fH; ()170gA; ()171N; H173Q,S; H172P; S184Q,P; E185S; S186A,E,P; L187A; ()187aS; T190P,A; D193S; A194S,T; M195T,V,L; N198A,V; G200V; S201D,E ()201eT; S202A; D203R,K,S; P203aV,T; Q204E,S,A,V; T205E; I211L,V; P215A; L220N; Q223D,H; A232T; D233E; S235Y,L,T; N236Y; L237F; I238L,M; A242P,S; E244D; I246V; ()251eE,Q; N256D; P260A,H; A264R,I; Q265A; E267D; G270Y,A,L; L332F; D271N; D273K; F275Y; T278H; Y280A,P; Y283P; V287A,T; Q288L,I,F; Y292F; I293A,V; E302R,H; P304A; L332F; N336S; Q337T,S,G; M338I; I339V; A340P; S343A,F,I,L; F348Y; N349P; S352K; P360R; R362P; W364F; V365L,A,S; T366D,V,S; S367K,A; R368K; L369I; T370V; S373A; A374S; R375H; S383kQ,E; T387P; A396R; G404A; L409R; T411K; L412R; E417R; Y421F.

28. The phytase variant of claim 27, the model phytase of which is a phytase derived from *Agroclybe pediades*.

29. The phytase variant of claim 27, the model phytase of which is a phytase derived from *Agroclybe pediades* CBS 900.96.

30. The phytase variant of claim 29, the model phytase of which is the *Agroclybe pediades* phytase sequence of FIG. 5.

31. The phytase variant according to any of claims 1-2, which phytase variant comprises at least one of the following amendments: F24C; V27P; L31Y; I33C; S39H,Q; N40L; G42S; P43A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y; Y44N; D45S; F47Y; E51A,R; E58D,K,A; T61R; V62I; W75F; S78D; A80K; R81Q,E,G,A; S82T; R83A,I,Q,K; Q84Y,V,I; V88I; K901R,A; A115N; D116S; L118V; P119E; F120L; N123T,Q; S125M; H126S,V; Q127E,N; T128A,S; M132F,I,L; G143N; V148I; A151S; D153Y; Q154D,S,G; D158A; S159T; S160A; T161N; ()170fH; ()170gA; S171NG172P; E173Q,S; Q184S,P; E185S; E186A,P; G187A; ()187aS; T190P,A; N193S; N194S,T; M195T,V,L; N198A,V; V200G; D201E; ()201eT; G202S,A; D203R,K,S; ()203aV,T; E204Q,S,A,V; S205E; V211L; N215A,P; L220N; A223D,H; S232T; D233E; L235Y,T; T236Y,N; L237F; M238L; P242S; L246V; ()251eE,Q; A260H; V264R,I; S265Q,A; E267D; Y270A,L,G; D271N; D273K; F275Y; G278T,H; P280A; A283P; T287A; Q288L,I,F; Y292F; V293A; G302R,H; A304P; N336S; T337Q,S,G; M338I; V339I; P340A; A343S,F,I,L; F348Y; N349P; A352K; E360R; R362P; W364F; V365L,A,S; D366V,S; S367K,A; L369I; S373A; G374A,S; ()383kQ,E; E387P; A396R; G404A; V409R; E411K,T; L412R; E417R; Y421F; A431E.

32. The phytase variant of claim 31, the model phytase of which is a phytase derived from *Peniophora lycii*.

33. The phytase variant of claim 32, the model phytase of which is a phytase derived from *Peniophora lycii* CBS 686.96.

34. The phytase variant of claim 33, the model phytase of which is the *Peniophora lycii* phytase sequence of FIG. 6.

35. A phytase polypeptide which comprises a phytase variant according to any of the previous claims.

36. A DNA construct comprising a DNA sequence encoding a phytase variant according to any one of claims 1-34.

37. A recombinant expression vector which comprises a DNA construct according to claim 36.

38. A host cell which is transformed with a DNA construct according to claim 36 or a vector according to claim 37.

39. A process for preparing a phytase variant, the process comprising culturing the host cell according to claim 38 under conditions permitting the production of the phytase variant, and recovering the phytase from the culture broth.

40. A feed or food comprising at least one phytase variant of any of claims 1-34.
41. A process for preparing a feed or food according to claim 40, wherein the at least one phytase variant is added to the food or feed components.
42. A composition comprising at least one phytase variant of any of claims 1-34.
43. The composition according to claim 42 suitable for use in food or feed preparations.
44. The composition according to any of claims 42-43, which is an animal feed additive.
45. A process for reducing phytate levels in animal manure comprising feeding an animal with an effective amount of the feed according to claim 40 or obtainable according to claim 41.
46. Use of the phytase variant of any of claims 1-34; or the composition of any of claims 42-43 for liberating phosphorous from a phytase substrate.
47. A transgenic plant or plant part which is capable of expressing a phytase variant according to any one of claims 1-34.
48. A modified phytase comprising a mutation in an amino acid sequence of a phytase, wherein the modified phytase has phytase activity and the mutation is at one or more positions selected from the group consisting of: 45, 61, 118, 203, 203a, 340, 360, 366, and 411, wherein each position corresponds to the position of the amino acid sequence of the mature *P. lycii* phytase (SEQ ID NO: 7).
49. The modified phytase of claim 48, wherein the mutation is selected from the group consisting of: 45D,S; 61R; 118V,L; 203R,K,S; 203aV,T; 340P,A; 360R; 366D,S,V; and 411K,T.
50. The modified phytase of claim 48, wherein the phytase is an ascomycete phytase.
51. The modified phytase of claim 50, wherein the phytase is an *Aspergillus* phytase.
52. The modified phytase of claim 51, wherein the phytase is an *Aspergillus ficuum*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, or *Aspergillus terreus* phytase.
53. The modified phytase of claim 52, wherein the phytase is an *Aspergillus terreus*, CBS 116.46 phytase.
54. The modified phytase of claim 48, wherein the phytase is a *Myceliophthora thermophila*, *Talaromyces thermophilus*, or *Thermomyces lanuginosus* phytase.
55. The modified phytase of claim 54, wherein the phytase is a *Myceliophthora thermophila*, ATCC 34625 or ATCC 74340 phytase.
56. The modified phytase of claim 54, wherein the phytase is a *Talaromyces thermophilus*, ATCC 20186 or ATCC 74338 phytase.
57. The modified phytase of claim 54, wherein the phytase is a *Thermomyces lanuginosus*, NRRL B-21527 phytase.
58. The modified phytase of claim 48, wherein the phytase is an ascomycete consensus phytase sequence.
59. The modified phytase of claim 48, wherein the phytase is a basidiomycete phytase.
60. The modified phytase of claim 59, wherein the phytase is a *Agrocybe pediades*, *Paxillus involutus*, *Peniophora lycii*, or *Trametes pubescens* phytase.

61. The modified phytase of claim 60, wherein the phytase is a Paxillus involutus, CBS 100231 phytase.

62. The modified phytase of claim 61, wherein the phytase is a Paxillus involutus, CBS 100231 Phy-A2 phytase.

61. The modified phytase of claim 60, wherein the phytase is a Trametes pubescens, CBS 100232 phytase.

64. A feed or food comprising a modified phytase of claim 48.

65. A composition comprising a modified phytase of claim 48.

66. A process for reducing phytate levels in animal manure comprising feeding an animal with an effective amount of the feed of claim 64.



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TITLE: Modified phytases

DATE-ISSUED: May 21, 2002

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US-CL-CURRENT: 435/196; 424/94.6

## CLAIMS:

What is claimed is:

1. A modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.

2. A modified *Aspergillus fumigatus* phytase according to claim 1, further comprising an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.

3. A modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the modified *Aspergillus fumigatus* phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations thereof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an *Aspergillus niger* phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.

4. A modified phytase according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

5. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ala.

6. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Val.
7. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Leu.
8. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ile.
9. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Thr.
10. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Asn.
11. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Gly.
12. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.
13. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.
14. A modified phytase according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.
15. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.
16. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.
17. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.
18. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.
19. A modified phytase according to claim 3 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.
20. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.
21. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.
22. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.
23. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.
24. A food or feed composition comprising a modified phytase of claim 1.